Volume 281, number 1,2, 9–19 FEBS 09577 April 1991

ADONIS 001457939100287J

Review Letter

DNA damage by oxygen-derived species

Its mechanism and measurement in mammalian systems

Barry Halliwell[1] and Okezie I. Aruoma[2]

[1]*Division of Pulmonary Medicine, UC Davis Medical Center, Sacramento, CA 95817, USA* and [2]*Department of Biochemistry, University of London King's College, Strand Campus, London WC2R 2LS, UK*

Received 11 December 1990; revised version received 22 January 1991

When cells are exposed to oxidative stress, DNA damage frequently occurs. The molecular mechanisms causing this damage may include activation of nucleases and direct reaction of hydroxyl radicals with the DNA. Several oxygen-derived species can attack DNA, producing distinctive patterns of chemical modification. Observation of these patterns and measurement of some of the products formed has been used to determine the role of different oxygen-derived species in DNA cleavage reactions, to assess the extent of oxidative damage to DNA in vivo and to investigate the mechanism of DNA damage by ionizing radiation and chemical carcinogens.

DNA; Mutation; Oxygen radical; Hydroxyl radical; Oxidative stress

1. INTRODUCTION

It is well-established that aerobic organisms constantly produce small amounts of reactive oxygen species[1], including superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), the latter being generated by the enzyme myeloperoxidase in neutrophils [1–3]. Exposure of living organisms to background levels of ionizing radiation leads to homolytic fission of oxygen–hydrogen bonds in water to produce hydroxyl radical, $^{\cdot}OH$ [4]. Hydroxyl radical can also be generated when H_2O_2 comes into contact with certain transition metal ion chelates, especially those of iron and copper [5]. In general, the reduced forms of these metal ions (Fe^{2+}, Cu^{+}) produce $^{\cdot}OH$ at a faster rate upon reaction with H_2O_2 than the oxidized forms (Fe^{3+}, Cu^{2+}) and so reducing agents such as $O_2^{\cdot-}$ and ascorbic acid can often accelerate $^{\cdot}OH$ generation by metal ion/H_2O_2 mixtures [5]. However, both Cu^{2+} [6,7] and certain Fe^{3+}-complexes (especially Fe^{3+}-nitrilotriacetic acid) do generate some $^{\cdot}OH$ upon raction with H_2O_2 [8–10]. In the case of ferric-NTA complexes, $O_2^{\cdot-}$ is somehow involved in the $^{\cdot}OH$ formation in the presence of H_2O_2, since it is almost completely inhibited by the $O_2^{\cdot-}$-scavenging enzyme superoxide dismutase [9,10].

Aerobes have evolved antioxidant defences to protect themselves against the reactive oxygen species[1] generated in vivo. These defences include enzymes (such as superoxide dismutase, catalase and glutathione peroxidase), low molecular mass agents (examples being α-tocopherol and ascorbic acid) and proteins that bind metal ions in forms unable to accelerate free radical reactions [1,2,11–13]. *Oxidative stress* results when reactive oxygen species are not adequately removed. This can happen if antioxidants are depleted and/or if the formation of reactive oxygen species is increased beyond the ability of the defences to cope with them [2].

Subjecting cells to oxidative stress can result in severe metabolic dysfunctions, including peroxidation of membrane lipids, depletion of nicotinamide nucleotides, rises in intracellular free Ca^{2+} ions, cytoskeletal disruption and DNA damage. The latter is often measured as formation of single-strand breaks, double strand breaks or chromosomal aberrations. Methods for measuring DNA strand breaks have recently been discussed [14–16]. Indeed, DNA damage has been almost invariably observed in a wide range of mammalian cell types exposed to oxidative stress [16–44]. The systems used to impose oxidative stress upon cells have included exposure to elevated oxygen concentrations [42], incubation with enzymes that generate reactive oxygen species (such as xanthine oxidase plus its substrates, xanthine or hypoxanthine [18,22–27]),

Correspondence address: B. Halliwell, Division of Pulmonary Medicine, UC Davis Medical Center, 4301 X Street, Sacramento, CA 95817, USA

[1] '*Reactive oxygen species*' is a collective term used in the biomedical literature that includes oxygen-containing radicals (such as $O_2^{\cdot-}$, $^{\cdot}OH$, $RO^{\cdot}$, $RO_2^{\cdot}$) and non-radical species that can produce oxygen-containing radicals during their reactions (H_2O_2, O_3, singlet $O_2\,^1\Delta g$, HOCl). The term 'reactive' is relative; for example, $O_2^{\cdot-}$ is very much less chemically reactive than is $^{\cdot}OH$.

direct addition to the cells of H_2O_2 [18,28–32,43,44, 44a,b], of organic hydroperoxides [33–36,44a,45], or of compounds whose metabolism by the cell results in increased intracellular generation of O_2^- and H_2O_2 (such as paraquat and menadione [19,21]), and co-incubation of the cells with activated phagocytes such as macrophages [38] and neutrophils [18,39–41]. Activated neutrophils and macrophages generate O_2^- and H_2O_2: in addition, neutrophils produce HOCl [3,46]. Neutrophils do not produce $^{\bullet}OH$ unless a source of transition metal ions is added to the incubation mixture, i.e. neutrophils do not themselves appear to contain any form of metal ion catalyst that will convert H_2O_2 into $^{\bullet}OH$ [47–49]. Oxidative stress [50–54] and DNA damage [51] also occur when some mammalian cells are exposed to tumor necrosis factor. The DNA damage produced in human cells by exposure to cigarette smoke [55,55a,56], asbestos [57,58], ozone [60,61] or to certain carcinogenic metals, such as nickel [59], has also been suggested to involve reactive oxygen species.

2. POSSIBLE MECHANISMS OF DNA DAMAGE INDUCED BY OXIDATIVE STRESS

Why does oxidative stress cause DNA damage? In the case of externally-generated reactive oxygen species (e.g. when cells are incubated with H_2O_2, activated phagocytes or xanthine oxidase plus its substrates) damage is usually inhibited by adding catalase, showing that H_2O_2 is needed. Superoxide dismutase (SOD) does not usually inhibit much, which could mean either that O_2^- is not involved in the DNA damage, or that SOD does not enter cells easily. That the latter interpretation is correct in at least one cell system is shown by the observations that SOD can protect hepatocytes from the toxicity of H_2O_2 or *t*-butylhydroperoxide under conditions where it does enter the cells [62,63]. However, neither O_2^- nor H_2O_2 undergoes *any* chemical reaction with DNA, as measured by strand breakage [64–66] or by chemical changes in the deoxyribose, purines or pyrimidines [9,67,68]. Hence, DNA damage by oxidative stress *cannot* involve direct attack of O_2^- or of H_2O_2 upon the DNA.

Two explanations of the DNA damage have been advanced (Fig. 1). First, it is possible that the damage is due to $^{\bullet}OH$ radical formation [30]. Thus, it is envisaged that H_2O_2, which crosses biological membranes easily [69], can penetrate to the nucleus and react with ions of iron or copper to form $^{\bullet}OH$. Because of the high reactivity of $^{\bullet}OH$ and its resultant inability to diffuse significant distances within the cell [69], this mechanism is only feasible if the $^{\bullet}OH$ is generated from H_2O_2 by reaction with metal ions bound upon or very close to the DNA. One possibility is that these metal ions might always be present bound to the DNA in vivo. For example, copper ions are thought to be present in chromosomes [70] and copper ions are very effective in promoting H_2O_2-dependent damage to isolated DNA [6,7] and to DNA within chromatin [71] in vitro. A second possibility is that the metal ions might be released within the cell as a result of oxidative stress, and then bind to the DNA [72]. Thus, just as oxidative stress causes rises in intracellular free Ca^{2+}, it may cause rises in intracellular free iron and/or copper ions that could bind to DNA and make it a target for oxidative damage [72–74]. It has recently been shown that some chelators of the carcinogenic metal nickel [59] also react with H_2O_2 to cause $^{\bullet}OH$-dependent damage to isolated DNA. Mixtures of cobalt(II) ions and H_2O_2 which are thought to produce $^{\bullet}OH$ [75], again damaged DNA in a way characteristic of attack by $^{\bullet}OH$ (Nackerdien, Rao, Halliwell, and Dizdaroglu, in preparation.

A second explanation of the ability of oxidative stress to cause DNA damage is that it triggers off a series of metabolic events within the cell [76,77] that lead to activation of nuclease enzymes, which cleave the DNA backbone. There has been much debate recently concerning the suggestion that oxidative stress causes rises in intracellular free Ca^{2+}, which might fragment DNA by activating Ca^{2+}-dependent endonucleases [21,37,78] in a mechanism resembling that of apoptosis ('programmed cell death'). An example of apoptosis is the killing of immature thymocytes by glucocorticoid hormones, which activate a self-destructive process that apparently involves Ca^{2+}-dependent DNA fragmentation [79,80].

These two mechanisms (DNA damage by $^{\bullet}OH$ or by activation of nucleases) are not mutually exclusive, i.e. they could both take place. Indeed, there is evidence consistent with both of them. Their relative importance may depend on the cell type used and on how the oxidative stress is imposed. For example, chelating agents that bind iron ions into chelates unable to generate $^{\bullet}OH$ (such as desferrioxamine [81], desferrithiocin [18], or phenanthroline [82]) can often protect cells against DNA damage and other toxic effects of oxidative stress [30,31,34,38,73,83–85]. The effects of desferrioxamine are variable, since in general it does not cross cell membranes readily, although it appears to enter some cell types (such as hepatocytes) more readily than it enters others. Jonas et al. [29] showed that the toxicity of H_2O_2 to epithelial cells is greatly diminished at 4°C but it can be increased again by adding ascorbic acid: this effect is not seen if cells are pre-treated with desferrioxamine. Their observations are consistent with a mechanism of cell damage that depends on H_2O_2 and reduced iron ions: at high temperatures normal metabolism may provide a reductant (such as O_2^-), whereas at low temperatures ascorbate can replace it. Suggestive evidence that Fenton-type reactions can occur within bacterial cells has been presented [87–89], although this is not necessarily relevant to mammalian systems. 8-Hydroxyguanine (8-OH-Gua) was increased in amount in the DNA of P388 D1 cells after exposure

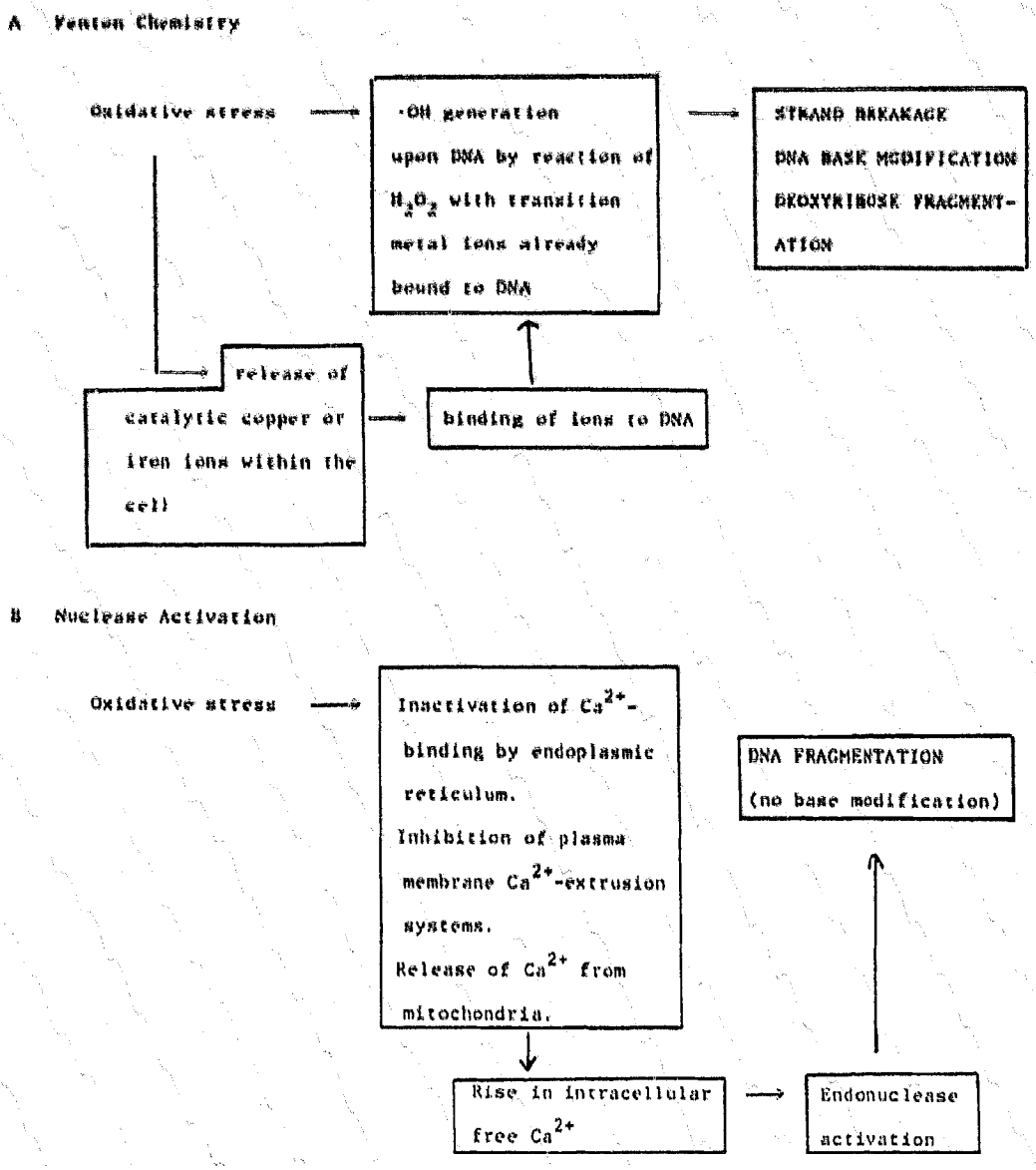

Fig. 1. Hypotheses to explain DNA damage resulting from exposing cells to oxidative stress.

to H_2O_2 [18]: 8-OH-Gua can arise from attack of 'OH upon guanine (see next section) but should not be produced as a result of nuclease action. DNA isolated from P388 D1 cells after exposing them to H_2O_2 did not show a regular pattern of fragmentation, such as might be expected from nuclease attack [18]. Increases in 8-OH-Gua have also been observed in DNA from other cells subjected to oxidative stress [90,91]. Thymine glycol, another product that can result from attack of 'OH upon DNA (see next section) has been reported to be formed in the DNA of yeast cells after exposure to high concentrations of H_2O_2 [92] and in murine tumor cells after exposure to tumor necrosis factor [51]. Treatment of murine hybridoma cells with H_2O_2 caused a pattern of chemical changes in the DNA bases that is characteristic of attack by 'OH [93].

However, the evidence for metabolic changes produced in cells by oxidative stress is also strong [21,37,76-79]. Menadione and other quinones (which 'redox cycle' within cells to give O_2^- and H_2O_2) appear to produce DNA strand breaks in hepatocytes by Ca^{2+}-dependent activation of an endonuclease. DNA damage could be inhibited by preventing the rise in Ca^{2+} using Ca^{2+}-chelators [37,94]. Oxidative stress can also sometimes activate and/or cause changes in the subcellular location of PKC (protein kinase C) [95-97]. Exposure of mouse epidermal JB6 cells to H_2O_2 appeared to cause a Ca^{2+}-dependent translocation of PKC to the plasma membrane [95] whereas menadione activated PKC both in these cells and in rat hepatocytes [95,96] without producing translocation. Cantoni et al. [98] found that the Ca^{2+}-chelator quin 2 inhibited

H_2O_2-induced DNA strand breakage in CHO cells, although it did not inhibit iron ion-dependent 'OH generation in vitro under their reaction conditions (its effect on copper ions was not examined). Of course, even if an iron ion-quin 2 complex is capable of catalyzing 'OH formation, it could still protect by removing metal ions from the vicinity of the DNA, so that any 'OH generated no longer attacks this molecule. Nicotera et al. [45] found that desferrioxamine prevented a sustained rise in intracellular free Ca^{2+} in hepatocytes exposed to *t*-butylhydroperoxide.

Fig. 2 shows that both types of experimental result may be accommodated by proposing that changes in the availability of calcium ions may depend upon, or give rise to, changes in the availability of iron or copper ions. Clearly, attempting to elucidate the mechanism of DNA damage by the use of free radical scavengers or metal ion chelators added to the outside of cells is unlikely to give unambiguous answers. Let us see what can be learned from the techniques of molecular biology and analytical chemistry.

3. REACTIVE OXYGEN SPECIES AS MUTAGENS AND CARCINOGENS

Oxidative stress, imposed by a variety of mechanisms (including increased O_2 concentrations [98a]), has been convincingly shown to be mutagenic to bacteria [69,99–104]. For example, *E. coli* mutants lacking SOD activity show greatly-enhanced rates of spontaneous mutation [99]. Similar mutagenic effects have been shown in a range of mammalian cell types [42,105–107] subjected to oxidative stress. Moraes et al. [108] studied the pattern of mutations obtained in a gene of a shuttle plasmid when simian cells transfected with this plasmid were exposed to H_2O_2. Both single base changes and deletions were observed. The majority of base changes were at GC base pairs, the GC→AT base transition being predominant. Treatment of the plasmid with H_2O_2 in vitro before transfection did not produce an increased number of mutations (unless iron ions were added [109]), consistent with the inability of H_2O_2 to react directly with DNA, as demonstrated by chemical studies [9,64–68].

Can mutations induced by oxidative stress lead to cancer? Ionizing radiation is well-known to be both mutagenic and carcinogenic [4,110,111]. Since much of the cell damage caused by such radiation involves 'OH production by homolytic fission of the oxygen-hydrogen bonds in water, then 'OH can probably be classified as a complete carcinogen. Base-pair changes and some frameshifts are the commonest mutations observed in cells exposed to ionizing radiation [110,111]. Chemical changes in the DNA bases, single- and double-strand breaks and enhanced expression of certain proto-oncogenes [4,112,113] have also been observed. However, the precise relationship between these different events and the development of cancer is uncertain. Thus, the chemical changes in DNA might themselves somehow lead to cancer [114]. An unrepaired lesion in DNA might be by-passed in an error-prone fashion. Resynthesis of DNA after excision repair might conceivably introduce errors.

There are many steps between a healthy cell and a malignant tumor. Cancer biologists have often referred to at least three stages: initiation (an irreversible change in DNA), promotion (probably involving changes in gene expression) and progression (further changes in DNA leading to the eventual production of a malignant tumor). Both Zimmerman and Cerutti [115] and Weitzman et al. [116] showed that a clone of C3H mouse fibroblasts exposed to activated human neutrophils or to hypoxanthine plus xanthine oxidase underwent malignant transformation. Nassi-Calo et al. [117] showed that H_2O_2 also transformed these cells, an action prevented by the chelating agent *o*-phenanthroline.

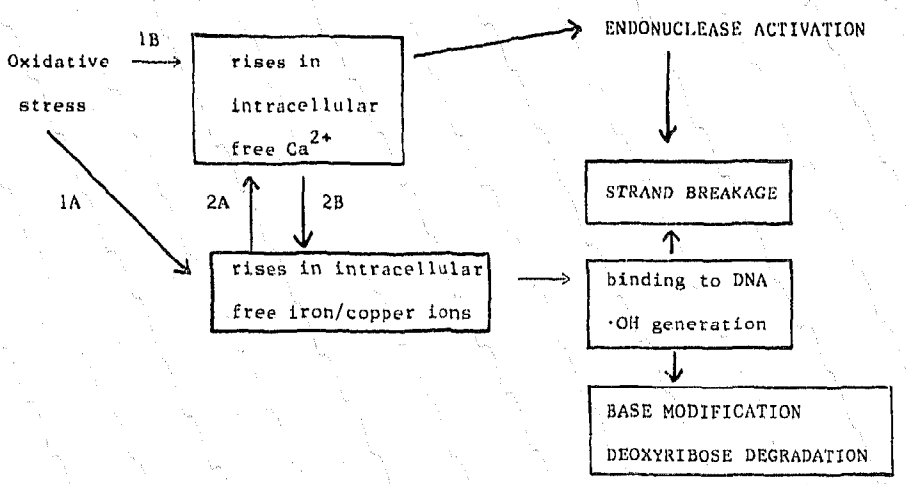

Fig. 2. A combined hypothesis. Rises in intracellular free iron or copper ion concentrations could be a consequence of rises in Ca^{2+} (1B, 2B) or vice versa (1A, 2A).

The ability of oxidative stress to induce transformation has also been shown in human lung fibroblasts [118], and the ability of H_2O_2 to cause pre-neoplastic changes in hamster tracheal explants has been described [119]. Increased expression of the proto-oncogenes c-*fos* and c-*myc* has been shown in several mammalian cell types exposed to oxidative stress [120–122]. Several tumor promoters, such as phorbol myristate acetate, are powerful activators of O_2^- and H_2O_2 production by phagocytes [123].

Although most attention has been paid in the literature to the action of reactive oxygen species as promoters of carcinogenesis [115,120,121,123], their ability to damage DNA and produce alterations in gene expression implies that they could be involved in all stages of carcinogenesis [124,127,129]. It has been argued [42,130–132] that continuous damage to DNA by free radical mechanisms is a significant cause of cancer in humans, an observation that might explain results of the epidemiological investigations, which show inverse correlations in humans between plasma concentrations of certain antioxidants and the incidence of cancer [131,133]. However, several antioxidants can alter the metabolism of procarcinogens, favoring metabolic pathways that do not result in formation of ultimate carcinogens [131,134]. Hence a protective effect of antioxidants does not *necessarily* mean that oxidative stress leads to the cancers in question. Of course, some carcinogens might act by imposing an oxidative stress on their target cells (Section 4), and reactive oxygen species have been claimed to be capable of converting some procarcinogens into ultimate carcinogens [134,135]. In addition, it must be borne in mind that a high plasma level of such antioxidants as ascorbic acid and vitamin E may simply be an index of a good diet, which protects against many diseases.

DNA damage resulting from oxidative stress (or from any other mechanism) need not necessarily lead to cancer. Low levels of damage may be efficiently repaired with a minimal risk of error. High levels of oxidative stress may lead to cell death, so that initiated cells do not remain in the organism. Thus, an intermediate level of damage is most likely to predispose to malignancy, which may explain the close association of chronic inflammation (involving phagocytic production of O_2^- and H_2O_2) with malignancy in such human diseases as ulcerative colitis, Crohn's disease and reflux esophagitis (reviewed in [127]). Cerutti et al. [122] showed that one difference between a clone of mouse epidermal cells that was promotable by xanthine/xanthine oxidase and a non-promotable clone was that the latter had lower levels of SOD and catalase and was more sensitive to killing by reactive oxygen species. Thus, increased antioxidant defences, by protecting against cell death resulting from oxidative stress, might conceivably (and ironically) sometimes lead to increased cancer [136,137].

4. CHARACTERIZATION AND GENETIC EFFECTS OF CHEMICAL CHANGES PRODUCED IN DNA BY REACTIVE OXYGEN SPECIES

What types of chemical change can be produced in DNA by reactive oxygen species? Superoxide and H_2O_2 do not react with DNA unless transition metal ions are present to allow $^{\cdot}OH$ formation [9,64–68].

Radiation chemists have carried out many studies of the effects of $^{\cdot}OH$, generated by ionizing radiation, upon DNA. This radical is so reactive that it can attack all components of the DNA (reviewed in [4,138–140]). Thus, $^{\cdot}OH$ abstracts hydrogen atoms from deoxyribose, giving sugar radicals that can fragment in various ways. Reactions of deoxyribose-derived radicals can lead to the release of purine and pyrimidine bases from the DNA (producing abasic sites), and to strand breaks. Some of the altered sugars that remain attached to DNA can be split to give strand breaks by incubation with alkaline solutions; these are the so-called 'alkali-labile sites' [4,15,141]. Chemical changes to the purine and pyrimidine bases have also been characterized in detail (reviewed in [138–140]). Thus, $^{\cdot}OH$ can add on to guanine residues at C-4, C-5, and C-8 positions. For example, addition of $^{\cdot}OH$ to C-8 of guanine produces a radical adduct that has several possible fates. It can be reduced to 8-hydroxy-7,8-dihydroguanine, oxidized to 8-hydroxyguanine, or undergo ring opening followed by one-electron reduction and protonation to give 2,6-diamino-4-hydroxy-5-formamidopyrimidine, usually abbreviated as FapyGua (Fig. 3). Similarly, $^{\cdot}OH$ can add on to C4, C5, or C8 of adenine residues: among other fates, the C-8 $^{\cdot}OH$ adduct radical can be converted into 8-hydroxyadenine by oxidation, or undergo ring-opening followed by one-electron reduction to give 5-formamido-4,6-diaminopyrimidine (FapyAde). Fig. 4 shows the structures of some of these compounds. Pyrimidines in DNA are also attacked to give multiple products. Thus, thymine can form *cis* and *trans* thymine glycols (5,6-dihydroxy-6-hydrothymines), 5-hydroxy-5-methylhydantoin, 5,6-dihydrothymine and 5-hydroxymethyluracil. Cytosine can form several products, including cytosine glycol and 5,6-dihydroxycytosine (Fig. 4).

When whole cells or isolated chromatin are exposed to ionizing radiation, cross-links can occur between DNA bases and amino acid residues in nuclear proteins [139,142–148]. Thus, thymine-tyrosine [145], thymine-aliphatic amino acid [143–149], and cytosine-tyrosine [146] links have been identified in isolated calf-thymus chromatin subjected to γ-irradiation. Treatment of chromatin with Fe^{2+}-chelates and H_2O_2 also produces DNA-protein cross-links [150] and such links have been detected in cellular DNA after exposure of the cells to ozone [60].

Molecular biologists have examined the likely

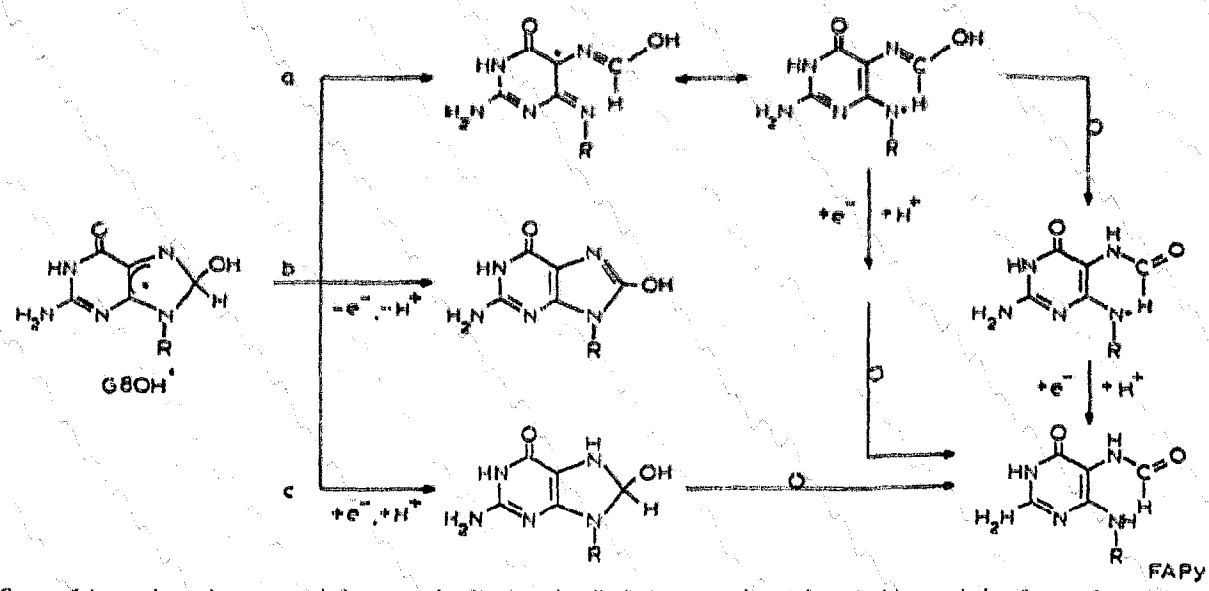

Fig. 3. Some of the products that can result from attack of hydroxyl radicals upon guanine. Adapted with permission from reference [138]. G8OH' is the radical formed by attack of 'OH upon C8 of guanine. It can undergo ring opening followed by reduction and protonation to give FapyGua (route a), oxidation to 8-OH-Gua (route b) or reduction to give 7,8-dihydro-8-hydroxyguanine, which can be converted to FapyGua (route c).

physiological effects of these various lesions in DNA. 8-Hydroxyguanine (and, by inference, 8-hydroxyadenine) might lead to mutations by inducing misreading of the base itself and of the adjacent bases [110,111,151,152]. Thymine glycol might have some mutagenic action and it can be lethal if not removed from the DNA before replication [110,111]. Ring-fragmented bases are thought to block DNA replication [110,111]. Abasic sites, which can result from direct attack of 'OH, can also be mutagenic in vivo [110,111].

It is clear that 'OH produces multiple changes in DNA whereas O_2^- and H_2O_2 have no effect, but the situation with other reactive oxygen species is less clear-cut at present. Singlet oxygen is able to produce limited strand breakage in isolated DNA [153,154], and its ability to modify the DNA bases is also limited [154a]. Thus, M. Dizdaroglu and H. Sies (personal communication) found small amounts of 8-OH-Gua and FapyGua but no other significant base changes in DNA exposed to singlet O_2 generated by the thermal decomposition of an endoperoxide. Exposure to illuminated Methylene blue causes formation of 8-hydroxyguanine [155] and of some strand breaks [156] in DNA but the species responsible was not identified, except for the

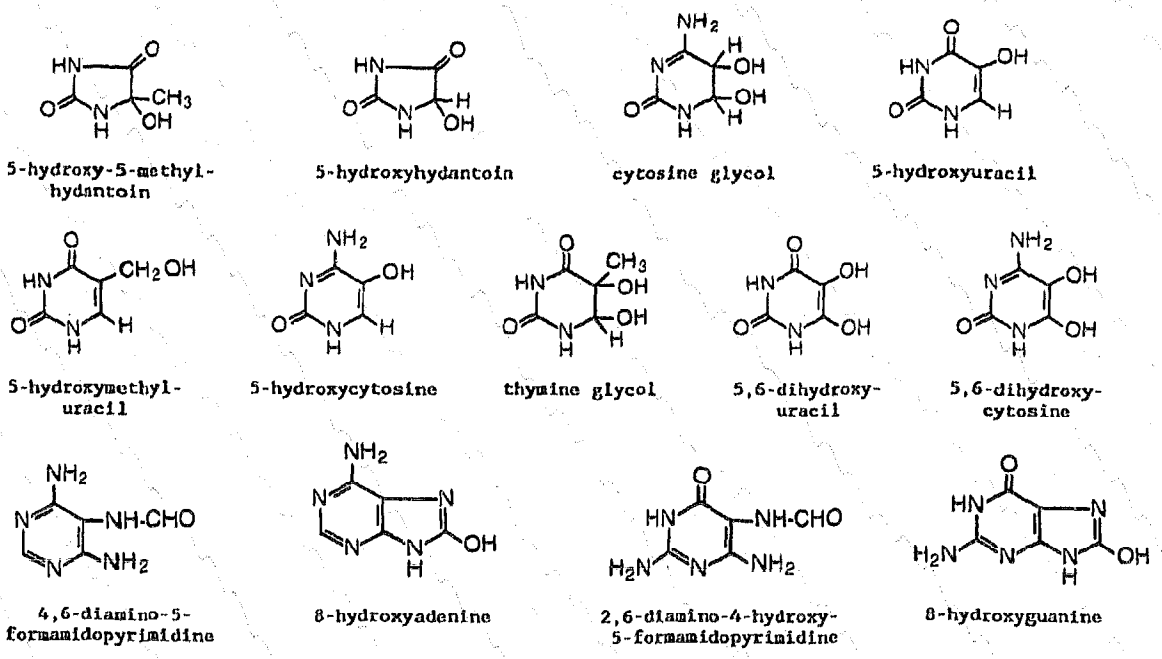

Fig. 4. Some of the end-products that result from attack of hydroxyl radicals upon the bases of DNA.

observation that scavengers of 'OH did not protect. However, the excited state of the photosensitizing dye Rose bengal has itself been claimed to cleave DNA [157] and, if 'OH were generated by Methylene blue bound to the DNA, then 'OH scavengers would not be expected to protect. Illuminated riboflavin, which generates singlet O_2, was reported not to produce 8-hydroxyguanine in DNA [158]. Thus, singlet O_2 certainly does not induce the extensive pattern of DNA base modification produced by 'OH.

Peroxidizing lipids have been reported to damage DNA [159-163a] but peroxidizing lipids produce a range of reactive oxygen species including 'OH, H_2O_2, singlet oxygen, peroxyl radicals, and alkoxyl radicals [69,164] and the exact contributions of these species to the DNA damage observed need to be determined [165,166]. Lipid peroxides also decompose to give a huge range of products [166-168] including carbonyl compounds, such as malondialdehyde and the unsaturated aldehyde 4-hydroxy-2-*trans* nonenal [168] which has been shown to be mutagenic to mammalian cells [169]. If these aldehydes are generated in the vicinity of DNA, they may be able to combine with it to form distinctive products [165,169]. Thus, malondialdehyde reacts with adenine, cytosine, and guanine [161,170a] and a guanine-MDA adduct has been identified in human urine [171]. The product of reaction of hydroxynonenal with deoxyguanosine has also been characterized [172].

Humans are constantly exposed to background levels of ionizing radiation, which will generate some 'OH in vivo. This radical may also arise by reaction of metal ions with H_2O_2 in vivo [5]. Thus, it is not surprising to find that repair systems have evolved to remove at least some of the lesions in DNA that can result from attack of 'OH and other reactive oxygen species (reviewed in [173,174]). Single-strand breaks are usually quickly repaired; indeed, they are generated as intermediates in the repair of other lesions (see below). 8-Hydroxyguanine is slowly removed from cellular DNA, but the repair mechanism is unknown [173]. Several lesions, including thymine glycol, are probably removed in human cells by action of a DNA glycosylase, which cuts the base-deoxyribose bond to give an abasic site. This site is recognized by an endonuclease activity (on the same enzyme), which nicks the strand at the abasic site. The damaged part of the strand is removed, followed by resynthesis of the DNA and re-joining of the strand by a DNA ligase enzyme. Glycosylases that recognize hydroxymethyluracil and ring-opened purines in DNA have also been described in mammalian cells [110,111,173,174].

Modified DNA bases and nucleosides (base-deoxyribose) have been detected in the urine of humans and other mammals. Thus, 8-hydroxyadenine, 7-methyl-8-hydroxyguanine, thymine glycol, thymidine glycol, hydroxymethyluracil, 8-hydroxyguanine and 8-hydroxydeoxyguanasine have been detected in mammalian urine [132,175-178]. The presence of these products in urine suggests that oxidative damage to the DNA bases does occur in vivo and that repair systems are active to cleave modified bases from DNA. However, it is possible that some excreted bases originate from the diet or from the metabolism of the gut flora, and that DNA released from dead and dying cells within an organism undergoes rapid oxidative damage (since cell disruption can increase free radical reactions [69,72,164]). Hence, one must be cautious in using the amounts of modified DNA bases excreted from the body as an index of the extent of repair of oxidative DNA damage in *healthy* cells.

5. MEASUREMENT OF BASE-DERIVED PRODUCTS AS A PROBE FOR THE MECHANISM AND EXTENT OF DNA DAMAGE

The development of an HPLC technique, coupled with highly-sensitive electrochemical detection, for the measurement of 8-hydroxy-deoxyguanosine has led to a series of pioneering studies in which measurement of this product has been used to gain information about free radical damage to DNA in intact cells and whole organisms [91,114,179-181]. Antibody techniques for the measurement of such products as 8-hydroxyguanine and thymine glycol have also long been available (e.g. see [182-184]). Ames et al. [175-177] have used the urinary excretion of products derived from guanine and thymine as an index of free radical damage to DNA in vivo, and have attempted to draw conclusions about changes in the rate of such damage as a function of age in mammals. The amount of 8-hydroxyguanine in the DNA from certain sub-populations of rat liver mitochondria was found to be considerably higher than that in nuclear DNA, leading to proposals about the role of mitochondria in aging and carcinogenesis [185,186]. Exposure of some cells to oxidative stress has been reported to lead to formation of 8-hydroxyguanine in the DNA [22,90,91,114]. For example, treatment of Ehrlich ascites cells with the carcinogen 4-nitroquinoline 1-oxide led to an increased content of 8-hydroxyguanine in DNA [187]. Intra-peritoneal injection of ferric-nitrilotriacetic acid (which reacts with H_2O_2 to give 'OH [9,10]) into rats produced a significant rise in the 8-hydroxyguanine content of kidney DNA [188]. Carcinogenic peroxisome proliferators [189,190], acetoxime [191], 2-nitropropane [191] and, in one study, a choline-deficient diet [191a], have all been reported to result in increased amounts of 8-hydroxyguanine in DNA in vivo in mammals.

These studies have certainly produced qualitative evidence for oxidative damage to DNA in vivo, although care must be used in interpreting the data [191b]. For example, nitroquinolines have been sug-

gested to form 8-hydroxyguanine in DNA by a mechanism that involves direct reaction of the ultimate carcinogen with DNA, rather than by oxygen radical generation [192]. One must also be extremely cautious in attempting to use measurement of any one product as a *quantitative* measure of DNA base damage by reactive oxygen species. When $^{\bullet}OH$ attacks DNA bases, radicals are formed that can react in various ways depending on the conditions used (Fig. 3 shows an example). Thus, attack of $^{\bullet}OH$ upon guanine can lead to formation of 8-hydroxyguanine by oxidation of the C-8 $^{\bullet}OH$ adduct radical, but this radical can lead to other products as well, depending on the reaction conditions. Thus, variable amounts of 8-hydroxyguanine can result from attack of the same amount of $^{\bullet}OH$ upon guanine in DNA, and so changes in 8-hydroxyguanine levels do not necessarily mean changes in the amount of free radical attack upon DNA. To take some examples, iron-ion dependent systems generating $^{\bullet}OH$ led to substantial formation of FapyGua as well as 8-hydroxyguanine in DNA [67], whereas systems containing copper ions and H_2O_2 greatly favored 8-hydroxyguanine production [6,71,193]. When isolated, mammalian chromatin was irradiated in aqueous suspension, the relative amounts of 8-hydroxypurines and formamidopyrimidines generated depended upon the radical environment provided by the gases used to saturate the aqueous solution [194]. For example, the presence of oxygen favoured the formation of 8-hydroxypurines [194,196]. Table I summarizes some of the results obtained. Products derived from pyrimidines can similarly be affected by changes in reaction conditions [195,196].

A complete characterization of damage to DNA by reactive oxygen species can be achieved by the technique of gas chromatography/mass spectrometry (reviewed in [194a,197,198]), which may be applied to DNA itself or to DNA-protein complexes such as chromatin. The DNA or chromatin are hydrolyzed and the products converted to volatile derivatives, which are separated by gas chromatography and identified by mass spectrometry. High sensitivity of detection can be achieved by operating the mass spectrometer in the selected ion monitoring (SIM) mode. In this mode, the mass spectrometer is set to monitor several ions derived by fragmentation of a particular product during the time at which this product is expected to emerge from the GC column. The GC/MS-SIM technique is being used in the authors' laboratories to examine the mechanism by which DNA is damaged in cells subjected to oxidative stress. Thus, if damage is due to $^{\bullet}OH$ generation, then products characteristic of $^{\bullet}OH$ attack should be detected (Fig. 1), as has been observed in murine hybridoma cells treated with H_2O_2 [93] and in primate tracheal epithelial cells exposed to ozone (Aruoma, Halliwell and Wu, in preparation). By contrast, cleavage of the DNA backbone by the action of nucleases should leave the purines and pyrimidines unaltered (Figs. 1 and 2). For studies on DNA modification, extraction of chromatin from cells for analysis is preferable to extraction of DNA, since it minimizes the loss of extensively-fragmented DNA, and of DNA that has become covalently cross-linked to protein.

Table I

How reaction conditions can alter the end products derived from attack of hydroxyl radicals generates by different systems upon purine bases in the DNA of isolated chromatin

Systems used to generate $^{\bullet}OH$	Ratios of	
	8-OH-Ade to FapyAde	8-OH-Gua to FapyGua
H_2O_2-metal ions' Air saturated solutions:		
H_2O_2/Fe^{3+}/ascorbate	1.1	5.9
H_2O_2/Fe^{3+}-EDTA	4.2	8.6
H_2O_2/Fe^{3+}-EDTA/ascorbate	0.5	2.2
H_2O_2/Fe^{3+}-NTA	1.5	8.3
H_2O_2/Fe^{3+}-NTA/ascorbate	1.2	5.3
H_2O_2/Cu^{2+}	18.6	48.4
H_2O_2/Cu^{2+}/ascorbate	11.1	31.5
Ionizing radiation: solutions saturated with		
Argon	0.55	0.57
Air	1.8	3.5
Nitrous oxide	0.8	0.75
Nitrous oxide and oxygen	3.4	4.5

Results were obtained with mammalian chromatin in aqueous suspension. Calculations by courtesy of Dr. M. Dizdaroglu. Data selected from [71,194].

GC/MS-SIM has been used to characterize the damage done to DNA by various reactive oxygen species. Hydroxyl radical appears to produce a uniquely-extensive pattern of base modifications (multiple products from all four bases), and this pattern seems to be a 'fingerprint' for $^{\bullet}OH$, i.e., it can be used to identify $^{\bullet}OH$ as a damaging species [6,9,68, 71,139,143,146,152,193,194,196,198,201]. For example, measurement of damage to the DNA bases by GC/MS-SIM has been used to show that the strand cleavage produced in isolated DNA by treatment with a copper-ion phenanthroline chelate probably involves $^{\bullet}OH$ [193], whereas DNA cleavage by a bleomycin-iron ion complex is not mediated by $^{\bullet}OH$ [199]. GC/MS has also been used to identify 8-OHGua, FApyAde, 8-OHAde and FApyGua in neoplastic tissues [200], to show that the damage done to the bases in isolated DNA by activated human neutrophils is most likely due to $^{\bullet}OH$ generated by reactions involving metal ions in the reaction mixture [201], to measure adducts of carcinogens with proteins in vivo in attempts to assess carcinogen exposure [202] and to characterize the changes produced in plasmid DNA by treating it with potassium permanganate [203]. The authors believe that such

'fingerprinting' of DNA damage is one way forward in investigating the role of reactive oxygen species in damaging DNA in vivo, and assessing the contribution that such species make to the mechanism of action of carcinogens and to the increased cumulative risk of cancer with age [132].

Acknowledgements: The authors are grateful to the Medical Research Council, Arthritis and Rheumatism Council and Association for International Cancer Research for research support. Drs. Lars Breimer and Miral Dizdaroglu are particularly thanked for their helpful comments upon the manuscript. We thank Dr. Miral Dizdaroglu and Prof. Helmut Sies for providing unpublished information. Dr. Aruoma was a visiting scientist (1988–89) at the National Institute of Standards and Technology, Gaithersburg, MD, USA.

REFERENCES

[1] Fridovich, I. (1989) Annu. Rev. Pharm. Tox. 23, 239–257.
[2] Sies, H., ed. (1991) Oxidative Stress: Oxidants and Antioxidants, Academic Press, New York.
[3] Weiss, S.J. (1989) New Engl. J. Med. 320, 365–376.
[4] Von Sonntag, C. (1987) The Chemical Basis of Radiation Biology, Taylor and Francis, London.
[5] Halliwell, B. and Gutteridge, J.M.C. (1990) Methods Enzymol. 86, 1–85.
[6] Aruoma, O.I., Halliwell, B., Gajewski, E. and Dizdaroglu, M. (1991) Biochem. J., 273, 601–604.
[7] Sagripanti, J.L. and Kramer, K.H. (1989) J. Biol. Chem. 264, 1729–1734.
[8] Inoue, S. and Kawanishi, S. (1987) Cancer Res. 47, 6522–6527.
[9] Aruoma, O.I., Halliwell, B., Gajewski, E. and Dizdaroglu, M. (1989) J. Biol. Chem. 264, 20509–20512.
[10] Gutteridge, J.M.C. (1990) Free Radical Res. Commun. 9, 119–125.
[11] Halliwell, B. and Gutteridge, J.M.C. (1990) Arch. Biochem. Biophys. 280, 1–8.
[12] Wayner, D.D.M., Burton, G.W. and Ingold, K.U. (1986) Biochim. Biophys. Acta 884, 119–123.
[13] Frei, B., England, L. and Ames, B.N. (1989) Proc. Natl. Acad. Sci. USA 86, 6377–6381.
[14] Birnboim, H.C. (1990) Methods Enzymol. 186, 550–555.
[15] Ahnström, G. (1988) Int. J. Radiat. Biol. 54, 695–707.
[16] Bhusate, L.L., Herbert, K.E. and Perrett, D. (1990) Biochem. Soc. Trans. 18, 676–677.
[17] Schraufstatter, I.U., Hinshaw, D.B., Hyslop, P.A., Spragg, R.G. and Cochrane, C.G. (1986) J. Clin. Invest. 77, 1312–1320.
[18] Schraufstatter, I.U., Hyslop, P.A., Jackson, J.H. and Cochrane, C.G. (1988) J. Clin. Invest. 82, 1040–1050.
[19] Eklow-Lastbom, L., Rossi, L., Thor, H. and Orrenius, S. (1986) Free Radical Res. Commun. 2, 57–68.
[20] Starke, P.E., Hock, J.B. and Farber, J.L. (1986) J. Biol. Chem. 261, 3006–3012.
[21] Orrenius, S., McConkey, D.J., Bellomo, G. and Nicotera, P. (1989) Trends Pharm. Sci. 10, 281–285.
[22] Weitberg, A.B. (1989) Mutat. Res. 216, 197–201.
[23] Paul, L.A., Fulton, A.M. and Heppner, G.H. (1989) Mutat. Res. 215, 223–234.
[24] Phillips, B.J., James, T.E.B. and Anderson, D. (1984) Mutat. Res. 126, 265–271.
[25] Iwata, K., Shibuya, H., Ohkawa, Y. and Inui, N. (1984) Toxicol. Lett. 22, 75–81.
[26] Hall, A.H. Jr., Eanes, R.Z., Waymack, P.P., Jr. and Patterson, R.M. (1988) Mutat. Res. 198, 161–168.
[27] Carson, D.A., Seto, S. and Wasson, D.B. (1986) J. Exp. Med. 163, 746–751.
[28] Olson, M.J. (1988) J. Tox. Environ. Hlth. 23, 407–423.
[29] Jonas, S.K., Riley, P.A. and Willson, R.L. (1989) Biochem. J. 264, 651–655.
[30] Mello Filho, A.C., Hoffmann, M.E. and Meneghini, R. (1984) Biochem. J. 218, 273–275.
[31] Kleiman, N.J., Wang, R.R. and Spector, A. (1990) Mutat. Res. 240, 35–45.
[32] Link, E.M. and Riley, P.A. (1988) Biochem. J. 249, 391–399.
[33] Ochi, T. (1989) Mutat. Res. 212, 243–248.
[34] Coleman, J.B., Gilfor, D. and Farber, J.L. (1986) Mol. Pharmacol. 30, 261–262.
[35] Nakayama, T., Kaneko, M. and Kodama, M. (1986) Agric. Biol. Chem. 50, 261–262.
[36] Norris, K.H. and Hornsby, P.J. (1988) in: Oxygen Radicals in Biology and Medicine (Simic, M.G., Taylor, K.A., Ward, J.F. and von Sonntag, C., eds.) Plenum, New York, pp. 461–466.
[37] Dypbukt, J.M., Thor, H. and Nicotera, P. (1990) Free Radical Res. Commun. 8, 346–354.
[38] Chong, Y.C., Heppner, G.H., Paul, L.A. and Fulton, A.M. (1989) Cancer Res. 49, 6652–6657.
[39] Weitberg, A.B., Weitzman, S.A., Destrempes, M., Latt, S.A. and Stossel, T.P. (1983) New Engl. J. Med. 308, 26–29.
[40] Weitberg, A.B., Weitzman, S.A., Clark, E.P. and Stossel, T.P. (1985) J. Clin. Invest. 75, 1835–1841.
[41] Shacter, E., Beecham, E.J., Covey, J.M., Kohn, K.W. and Potter, M. (1988) Carcinogenesis 9, 2297–2304.
[42] Gille, J.J.P., Mullaart, E., Vijg, J., Leyva, A.L., Arwert, F. and Joenje, M. (1989) Mutat. Res. 219, 17–28.
[43] Cantoni, O., Cattabeni, E., Stocchi, V., Meyn, R.E., Cerutti, P. and Murray, D. (1989) Biochim. Biophys. Acta 1014, 1–7.
[44] Prise, K.M., Davies, S. and Michael, B.D. (1989) Int. J. Rad. Biol. 55, 583–592.
[44a] Sanstrom, B.E. and Marklund, S.L. (1990) Biochem. J. 271, 17–23.
[44b] Spragg, R.G. (1991) Am. J. Respir. Cell. Mol. Biol. 4, 4–10.
[45] Nicotera, P., McConkey, D., Svensson, S.A., Bellomo, G. and Orrenius, S. (1988) Toxicology 52, 55–63.
[46] Curnutte, J.T. and Babior, B.M. (1987) Adv. Human Genet. 6, 229–297.
[47] Thomas, P.J., Shirley, P.S., Hedrick, C.C. and De Chatelet, L.R. (1986) Biochemistry 25, 8042–8048.
[48] Britigan, B.E., Coffman, T.J. and Buettner, G.R. (1990) J. Biol. Chem. 265, 2650–2656.
[49] Kaur, H., Fagerheim, I., Grootveld, M., Puppo, A. and Halliwell, B. (1988) Anal. Biochem. 172, 360–367.
[50] Larrick, J.W. and Wright, S.C. (1990) FASEB J. 4, 3215–3223.
[51] Zimmerman, R.J., Chan, A. and Leadon, S.A. (1987) Cancer Res. 49, 1644–1648.
[52] Wong, G. and Goeddel, D. (1988) Science 242, 941–944.
[53] Shaffer, J.B., Treanor, C.P. and Del Vecchio, P.J. (1990) Free Rad. Biol. Med. 8, 497–502.
[54] Hauser, G.J., McIntosh, J.K., Travis, W.D. and Rosenbery, S.A. (1990) Cancer Res. 50, 3503–3508.
[55] Borish, E.T., Cosgrove, J.P., Church, D.F., Deutsch, W.A. and Pryor, W.A. (1985) Biochem. Biophys. Res. Commun. 133, 780–786.
[55a] Kiyosawa, H., Suko, M., Okudaira, H., Murata, K., Miyamoto, T., Chung, M.H., Kasai, H. and Nishimura, S. (1990) Free Radical Res. Commun. 11, 23–27.
[56] Nakayama, T., Kaneko, M., Kodama, M. and Nagata, C. (1985) Nature 314, 462–464.
[57] Weitzman, S.A. and Graceffa, P. (1984) Arch. Biochem. Biophys. 228, 373–376.
[58] Jackson, J.H., Schraufstatter, I.U., Hyslop, P.A., Vosbeck, K., Sauerheber, R., Weitzman, S.A. and Cochrane, C.G. (1987) J. Clin. Invest. 80, 1090–1095.
[59] Kawanishi, S., Inoue, S. and Yamamoto, K. (1989) Carcinogenesis 10, 2231–2235.
[60] Van der Zee, J., Van Beck, E., Dubbelman, T.M.A.R. and Van Steveninck, J. (1987) Biochem. J. 247, 69–72.
[61] Borek, C., Zaider, M., Ong, A., Mason, H. and Witz, G. (1986) Carcinogenesis 7, 1611–1613.

[62] Kyle, M.E., Nakae, D., Sakaida, I., Miccadei, S. and Farber, J.L. (1988) J. Biol. Chem. 263, 3784-3789.
[63] Nakae, D., Yoshiji, H., Amanuma, T., Kinugasa, T., Farber, J.L. and Konishi, Y. (1990) Arch. Biochem. Biophys. 279, 315-319.
[64] Brawn, M.K. and Fridovich, I. (1981) Arch. Biochem. Biophys. 206, 414-419.
[65] Lesko, S.A., Lorentzen, R.J. and Tso, P.O.P. (1980) Biochemistry 19, 3023-3028.
[66] Rowley, D.A. and Halliwell, B. (1983) Biochim. Biophys. Acta 761, 86-93.
[67] Aruoma, O.I., Halliwell, B. and Dizdaroglu, M. (1989) J. Biol. Chem. 264, 13024-13028.
[68] Blakely, W.F., Fuciarelli, A.F., Wegher, B.J. and Dizdaroglu, M. (1990) Radiat. Res. 121, 338-343.
[69] Halliwell, B. and Gutteridge, J.M.C. (1989) Free Radicals in Biology and Medicine, second edition, Clarendon Press, Oxford.
[70] Prutz, W.A., Butler, J. and Land, E.J. (1990) Int. J. Rad. Biol. 58, 215-234.
[71] Dizdaroglu, M., Rao, G., Halliwell, B. and Gajewski, E. (1991) Arch. Biochem. Biophys. 285, 317-324.
[72] Halliwell, B. (1987) FASEB J. 1, 358-364.
[73] Sakaida, I., Kyle, M.E. and Farber, J.L. (1990) Mol. Pharmacol. 37, 435-442.
[74] Starke, P.E., Gilbertson, J.D. and Farber, J.L. (1985) Biochem. Biophys. Res. Commun. 133, 371-379.
[75] Moorhouse, C.P., Halliwell, B., Grootveld, M. and Gutteridge, J.M.C. (1985) Biochim. Biophys. Acta 843, 261-268.
[76] Birnboim, H.C. and Kanabus-Kaminska, M. (1985) Proc. Natl. Acad. Sci. USA 82, 6820-6824.
[77] Birnboim, H.C. (1988) Biochem. Cell Biol. 66, 374-381.
[78] Farber, J.L. (1990) Chem. Res. Tox. 3, 503-508.
[78a] Orrenius, S., McConkey, D.J., Jones, D.P. and Nicotera, P. (1988) ISI Atlas Sci. Pharmacol. 1, 319-324.
[79] McConkey, D.J., Hartzell, P., Nicotera, P. and Orrenius, S. (1989) FASEB J. 3, 1843-1849.
[80] Arends, M.J., Morris, R.G. and Wyllie, A.H. (1990) Am. J. Path. 136, 593-608.
[81] Halliwell, B. (1989) Free Rad. Biol. Med. 7, 645-651.
[82] Halliwell, B. (1978) FEBS Lett. 96, 238-242.
[83] Meneghini, R. (1988) Mutat. Res. 195, 215-230.
[84] Kyle, M.E., Nakae, D., Sakaida, I., Serroni, A. and Farber, J.L. (1989) Biochem. Pharmacol. 38, 3797-3805.
[85] Starke, P.E. and Farber, J.L. (1985) J. Biol. Chem. 260, 10099-10104.
[86] Gannon, D.E., Varani, J., Phan, S.H., Ward, J.H., Kaplan, J., Till, G.O., Simon, R.H., Ryan, U.S. and Ward, P.A. (1987) Lab. Invest. 57, 37-44.
[87] Imlay, J.A. and Linn, S. (1988) Science 240, 1302-1309.
[88] Brandi, G., Cattabeni, F., Albano, A. and Cantoni, O. (1989) Free Radical Res. Commun. 6, 47-55.
[89] Hassett, D.J., Bean, K., Biswas, G. and Cohen, M.S. (1989) Free Radical Res. Commun. 7, 83-87.
[90] Floyd, R.A., Watson, J.J., Harris, J., West, M. and Wong, P.K. (1986) Biochem. Biophys. Res. Commun. 137, 841-846.
[91] Kasai, H., Crain, P.F., Kuchino, Y., Nishimura, S., Ootsuyama, A. and Tanooka, H. (1986) Carcinogenesis 7, 1849-1851.
[92] Kaneko, M., Leadon, S.A. and Ito, T. (1988) Mutat. Res. 207, 17-22.
[93] Dizdaroglu, M., Nackerdien, Z., Chao, B.C., Gajewski, E. and Rao, G. (1991) Arch. Biochem. Biophys, 285, 388-390.
[94] McConkey, D.J., Hartzell, P., Nicotera, P., Wyllie, A.H. and Orrenius, S. (1988) Toxicol. Lett. 42, 123-130.
[95] Larsson, R. and Cerutti, P. (1989) Cancer Res. 49, 5627-5632.
[96] Kass, G.E.N., Duddy, S.K. and Orrenius, S. (1989) Biochem. J. 260, 499-507.
[97] Gopalakrishna, R. and Anderson, W.B. (1989) Proc. Natl. Acad. Sci. USA 86, 6758-6762.
[98] Cantoni, O., Sestili, P., Cattabeni, F., Bellomo, G., Pou, S., Cohen, M. and Cerutti, P. (1989) Eur. J. Biochem. 182, 209-212.
[98a] Joenje, H. (1989) Mutat. Res. 219, 193-208.
[99] Touati, D. (1989) Free Radical Res. Commun. 8, 1-9.
[100] Moody, C.S. and Hassan, H.M. (1982) Proc. Natl. Acad. Sci. USA 79, 2855-2859.
[101] Storz, G., Christman, M.F., Sies, H. and Ames, B.N. (1987) Proc. Natl. Acad. Sci. USA 84, 8917-8921.
[102] Levin, D.E., Hollstein, M., Christman, M.F., Schwiers, E.A. and Ames, B.N. (1982) Proc. Natl. Acad. Sci. USA 79, 7445-7449.
[103] Barak, M., Ulitzur, S. and Merzbach, D. (1983) Mutat. Res. 121, 7-16.
[104] Abril, N. and Pueyo, C. (1990) Environ. Molec. Mutagen. 15, 184-189.
[105] Weitzman, S.A. and Stossel, T.P. (1981) Science 212, 546-548.
[106] Weitzman, S.A. and Stossel, T.P. (1984) Cancer Lett. 22, 337-342.
[107] Hsie, A.W., Recio, L., Katz, D.S., Lee, C.Q., Wagner, M. and Schenley, R.L. (1986) Proc. Natl. Acad. Sci. USA 83, 9616-9620.
[108] Moraes, E.C., Keyse, S.M. and Tyrrell, R.M. (1990) Carcinogenesis 11, 283-293.
[109] Moraes, E.C., Keyse, S.M., Pidoux, M. and Tyrrell, R.M. (1989) Nucleic Acids Res. 17, 8301-8312.
[110] Breimer, L.H. (1988) Br. J. Cancer 57, 6-18.
[111] Breimer, L.H. (1990) Mol. Carcinogenesis 3, 188-197.
[112] Ziegler-Skylakakis, K. and Andrea, U. (1987) Mutat. Res. 192, 65-67.
[113] Yamashina, K., Miller, B. and Heppner, G.H. (1986) Cancer Res. 46, 2396-2400.
[114] Floyd, R.A. (1990) Carcinogenesis 11, 1447-1450.
[115] Zimmerman, R. and Cerutti, P. (1984) Proc. Natl. Acad. Sci. USA 81, 2085-2087.
[116] Weitzman, S.A. Weitberg, A.B., Clark, E.P. and Stossel, T.P. (1985) Science 227, 1231-1233.
[117] Nassi-Calo, L., Mello-Filho, A.C. and Meneghini, R. (1989) Carcinogenesis 10, 1055-1057.
[118] Weitberg, A.B. and Corvese, D. (1990) Biochem. Biophys. Res. Commun. 169, 70-74.
[119] Radosevich, C.A. and Weitzman, S.A. (1989) Carcinogenesis 10, 1943-1946.
[120] Sherman, M.L., Datta, R., Hallahan, D.E., Weichselbaum, R.R. and Kufe, D.W. (1990) Proc. Natl. Acad. Sci. USA 87, 5663-5666.
[121] Shibanuma, M., Kuroki, T. and Nose, K. (1988) Oncogene 3, 17-21.
[122] Cerutti, P., Larsson, R., Krupitza, G., Muehlmatter, D., Crawford, D. and Amstad, P. (1989) Mutat. Res. 214, 81-88.
[123] Cerutti, P. (1985) Science 227, 375-381.
[124] Perchelett, J.P. and Perchellet, E.M. (1989) Free Rad. Biol. Med. 7, 377-408.
[125] Kensler, T.W. and Taffe, B.G. (1986) Adv. Free Rad. Biol. Med. 2, 347-387.
[126] Goldstein, B.D., Czerniecki, B. and Witz, G. (1989) Environ. Health Perspect. 81, 55-57.
[127] Weitzman, S.A. and Gordon, L.I. (1990) Blood 76, 655-663.
[128] Halliwell, B. and Gutteridge, J.M.C. (1989) Free Radicals in Biology and Medicine, Second Edition, Clarendon Press, Oxford, Chapter 8.
[129] Stich, H.F. and Anders, F. (1989) Mutat. Res. 214, 47-61.
[130] Totter, J.R. (1980) Proc. Natl. Acad. Sci. USA 77, 1763-1767.
[131] Ames, B.N. (1983) Science 221, 1256-1264.
[132] Ames, B.N. (1989) Free Radical Res. Commun. 7, 121-128.
[133] Gey, K.F., Stahelin, H.B. and Brubacher, G.B. (1988) in: Medical, Biochemical and Chemical Aspects of Free Radicals. In Proceedings of the 4th Biennial General Meeting of the Society for Free Radical Research (Hayaishi, O., Niki, E., Kondo, M. and Yoshikawa, T. eds.) Elsevier, Amsterdam, pp. 377-384.

[134] Gower, J.D. (1988) Free Rad. Biol. Med. 5, 95–111.
[135] Reed, G.A. (1987) Chem. Phys. Lipids 44, 127–148.
[136] Okamoto, H. (1985) Bioessays 2, 15–22.
[137] Boothman, D.A. and Pardee, A.B. (1989) Proc. Natl. Acad. Sci. USA 86, 4963–4967.
[138] Steenken, S. (1989) Chem. Rev. 89, 503–520.
[139] Dizdaroglu, M. (1986) Biotechniques 4, 536–546.
[140] Teoule, R. (1987) Int. J. Radiat. Biol. 51, 573–589.
[141] Beesk, F., Dizdaroglu, M., Schulte-Frohlinde, D. and Von Sonntag, C. (1979) Int. J. Rad. Biol. 36, 565–576.
[142] Mee, L.K. and Adelstein, S.J. (1981) Proc. Natl. Acad. Sci. USA 78, 2194–2198.
[143] Gajewski, E., Fuciarelli, A.F. and Dizdaroglu, M. (1988) Int. J. Radiat. Biol. 54, 445–459.
[144] Dizdaroglu, M. and Gajewski, E. (1989) Cancer Res. 49, 3463–3467.
[145] Dizdaroglu, M., Gajewski, E., Reddy, P. and Margolis, S.A. (1989) Biochemistry 28, 3625–3628.
[146] Gajewski, E. and Dizdaroglu, M. (1990) Biochemistry 29, 977–980.
[147] Cress, A.E. and Bowden, G.T. (1983) Radiat. Res. 95, 610–618.
[148] Oleinick, N.L., Chiu, S., Ramakrishman, N. and Xue, L. (1987) Br. J. Cancer 55 (suppl. VIII), 135–140.
[149] Gajewski, E., Fuciarelli, E.F. and Dizdaroglu, M. (1988) Int. J. Radiat. Biol. 54, 455–459.
[150] Lesko, S.O., Drocourt, J.L. and Yang, S.U. (1982) Biochemistry 21, 5010–5015.
[151] Kuchino, Y., Mori, F., Kasai, H., Inoue, H., Iwai, S., Miura, K., Ohtsuka, E. and Nishimura, S. (1987) Nature 327, 77–79.
[152] Wood, M.L., Dizdaroglu, M., Gajewski, E. and Essigmann, J.M. (1990) Biochemistry 29, 7024–7032.
[153] Di Mascio, P., Wefers, H., Do-Thi, H.P., Lafleur, M.V.M. and Sies, H. (1989) Biochim. Biophys. Acta 1007, 151–157.
[154] Blazek, E.R., Peak, J.G. and Peak, M.J. (1989) Photochem. Photobiol. 49, 607–613.
[154a] Muller, E., Boiteaux, S., Cunningham, R.P. and Epe, B. (1990) Nucleic Acids Res. 18, 5969–5973.
[155] Schneider, J.E., Price, S., Maidt, L., Gutteridge, J.M.C. and Floyd, R.A. (1990) Nucleic Acids Res. 18, 631–635.
[156] Epe, B., Hegler, J. and Wild, D. (1989) Carcinogenesis 10, 2019–2024.
[157] Peak, J.M., Peak, J.G., Foote, C.S. and Krinsky, N.I. (1984) J. Photochem. 25, 309–315.
[158] Floyd, R.A., West, M.S., Eneff, K.L. and Schneider, J.E. (1990) Free Rad. Biol. Med. 8, 327–330.
[159] Brambilla, G., Martelli, A. and Marinari, U.M. (1989) Mutat. Res. 214, 123–127.
[160] Kasai, H. and Nishimura, S. (1988) in: Medical, Biochemical and Chemical Aspects of Free Radicals (Hayaishi, O., Niki, E., Kondo, M. and Yoshikawa, T. eds.) Elsevier, Amsterdam, 1021–1023.
[161] Vaca, C.E., Wilhelm, J. and Harms-Ringdahl, M. (1988) Mutat. Res. 195, 137–149.
[162] Inouy, S. (1984) FEBS Lett. 172, 231–234.
[163] Hruszkewycz, A.M. and Bertgold, D.S. (1990) Mut. Res. 244, 123–128.
[163a] Hruszkewewycz, A.M. (1988) Biochem. Biophys. Res. Commun. 153, 191–197.
[164] Gutteridge, J.M.C. and Halliwell, B. (1990) Trends Biochem. Sci. 15, 129–135.
[165] Morita, J., Veda, K., Nakai, K., Baba, Y. and Komano, T. (1983) Agric. Biol. Chem. 47, 2977–2979.
[166] Frankel, E.N., Neff, W.E., Brooks, D.D. and Fujimoto, K. (1987) Biochim. Biophys. Acta 919, 239–244.
[167] Frankel, E.N. (1987) Chem. Phys. Lipids 44, 73–85.
[168] Esterbauer, H., Zollner, H. and Schaur, R.J. (1988) ISI Atlas Sci. Biochem. 1, 311–317.
[169] Brambilla, G., Sciaba, L., Faggin, P., Maura, A., Marinari, U.M., Ferro, M. and Esterbauer, H. (1986) Mutat. Res. 171, 169–176.
[170] Stone, K., Ksebati, M.B. and Marnett, L.J. (1990) Chem. Res. Tox. 3, 33–38.
[170a] Stone, K., Uzieblo, A. and Marnett, L.J. (1990) Chem. Res. Tox. 3, 467–472.
[171] Hadley, M. and Draper, H.H. (1990) Lipids 25, 81–85.
[172] Sodum, R.S. and Chung, F.L. (1988) Cancer Res. 48, 320–323.
[173] Breimer, L.H. (1991) Free Radical Res. Commun., in press.
[174] Doetsch, P.W., Hamilton, K.K., Rapkin, L., Okenquist, S.A. and Lenz, J. (1990) in: Ionizing Radiation Damage to DNA: Molecular Aspects (Wallace, S.S. and Painter, R.B. eds.) Wiley-Liss, New York, pp. 109–125.
[175] Catheart, R., Schwiers, E., Saul, R.L. and Ames, B.N. (1984) Proc. Natl. Acad. Sci. USA 81, 5633–5637.
[176] Adelman, R., Saul, R.L. and Ames, B.N. (1988) Proc. Natl. Acad. Sci. USA 85, 2706–2708.
[177] Fraga, C.C., Shigenaka, M.K., Park, J.W., Degan, P. and Ames, B.N. (1990) Proc. Natl. Acad. Sci. USA 87, 4533–4537.
[178] Stillwell, W.G., Xu, H.X., Adkins, J.A., Wishnok, J.S. and Tannenbaum, S.R. (1989) Chem. Res. Toxicol. 2, 94–99.
[179] Floyd, R.A., Watson, J.J., Wong, P.K., Altmiller, D.H. and Rickard, R.C. (1986) Free Radical Res. Commun. 1, 163–172.
[180] Floyd, R.A., West, M.S., Eneff, K.L., Schneider, J.E., Wong, P.K., Tingey, D.T. and Hogsett, W.E. (1990) Anal. Biochem. 188, 155–158.
[181] Floyd, R.A., West, M.S., Hogsett, W.E. and Tingey, D.T. (1989) Plant Physiol. 91, 644–647.
[182] Kasai, H. and Nishimura, S. (1986) Environ. Health Perspect. 67, 111–116.
[183] Kaneko, M. and Leadon, S.A. (1986) Cancer Res. 46, 71–75.
[184] Hubbard, K., Huang, H., Laspia, M.F., Ide, H., Erlanger, B.F. and Wallace, S.S. (1989) Radiat. Res. 118, 257–268.
[185] Richter, C., Park, J.W. and Ames, B.N. (1988) Proc. Natl. Acad. Sci. USA 85, 6465–6467.
[186] Richter, C. (1988) FEBS Lett. 241, 1–5.
[187] Kohda, K., Tada, M., Kasai, H., Nishimura, S. and Kawazoe, Y. (1986) Biochem. Biophys. Res. Commun. 139, 626–632.
[188] Umemura, T., Sai, K., Takagi, A., Hasegawa, R. and Kurokawa, Y. (1990) Carcinogenesis 11, 345–347.
[189] Kasai, H., Okada, Y., Nishimura, S., Rao, H.S. and Reddy, J.K. (1989) Cancer Res. 49, 2603–2605.
[190] Hussain, N.S., Conaway, C.C., Guo, N., Asaad, W. and Fiala, E.S. (1990) Carcinogenesis 11, 1013–1016.
[191] Takagi, A., Sai, K., Umemura, T., Hasegawa, R. and Kurokawa, Y. (1990) Jpn. J. Cancer Res. 81, 213–215.
[191a] Hinrichsen, L.I., Floyd, R.A. and Sudilovsky, O. (1990) Carcinogenesis 11, 1879–1881.
[191b] Hegi, M.E., Ulrich, D., Sagelsdorf, P., Richter, C. and Lutz, W.K. (1990) Mutat. Res. 238, 325–329.
[192] Kohda, K., Tada, M., Hakura, A., Kasai, H. and Kawazoe, Y. (1987) Biochem. Biophys Res. Commun. 149, 1141–1148.
[193] Dizdaroglu, M., Aruoma, O.I. and Halliwell, B. (1990) Biochemistry 29, 8447–8451.
[194a] Dizdaroglu, M. and Gajewski, E. (1990) Methods Enzymol. 186, 530–544.
[195] Tofigh, S. and Frenkel, K. (1989) Free Rad. Biol. Med. 7, 131–143.
[196] Fuciarelli, A.F., Wegher, B.J., Blakely, W.F. and Dizdaroglu, M. (1990) Int. J. Radiat. Biol. 58, 397–415.
[197] Dizdaroglu, M. (1985) Anal. Biochem. 144, 593–603.
[198] Dizdaroglu, M. (1991) Free Rad. Biol. Med., in press.
[199] Gajewski, E., Aruoma, O.I., Dizdaroglu, M. and Halliwell, B. (1991) Biochemistry, in press.
[200] Malins, D.C., Ostrander, G.K., Haimanot, R. and Williams, P. (1990) Carcinogenesis 11, 1045–1047.
[201] Jackson, J.H., Gajewski, E., Schraufstatter, I.U., Hyslop, P.A., Fuciarelli, A.F., Cochrane, C.G. and Dizdaroglu, M. (1989) J. Clin. Invest. 84, 1644–1649.
[202] Skipper, P.L. and Tannenbaum, S.R. (1990) Carcinogenesis 11, 507–518.
[203] Akman, S.A., Doroshow, J.H. and Dizdaroglu, M. (1990) Arch. Biochem. Biophys. 282, 202–205.